IN THE UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF ILLINOIS
EASTERN DIVISION

CELSIS IN VITRO, INC. a Maryland Corporation, *Plaintiff,* v. CELLZDIRECT, INC., a Delaware Corporation and wholly-owned subsidiary of INVITROGEN CORPORATION; and INVITROGEN CORPORATION, a Delaware Corporation. *Defendants.*	)	Case No. _____

DECLARATION OF STEPHEN C. STROM, PH.D.

I, Stephen C. Strom, Ph.D., submit this Declaration in support of Plaintiff Celsis IVT's Memorandum in Support of its Motion for Preliminary Injunction which discloses my opinions and qualifications. My opinions are based upon information presently available to me and I reserve the right to supplement or modify my opinions in response to additional information provided by the Defendants that may effect my opinions.

I. BACKGROUND

A. Qualifications

1. I am a Professor of Pathology at the University of Pittsburgh in Pittsburgh, Pennsylvania. Pathology is generally the science or the study of the origin, nature, and course of diseases.

2. In 1974 I earned my Bachelor of Science degree from Westmar College in Le Mars, Iowa, and in 1978 I earned my Ph.D. in Pharmacology from the University of Kansas Medical Center in Kansas City, Kansas.

3. From 1978-1982, I conducted my post-graduate studies as a Post-Doctoral Fellow under Dr. George Michalopoulos at the Duke University Medical Center in the Department of Pathology. Dr. Michalopoulos is one of the most prominent hepatocyte researchers in the world. During my post-graduate studies I concentrated my research on mutation induction, carcinogen metabolism, and DNA damage and repair in cultures of rat and human hepatocytes, as well as human fibroblasts.

4. From 1982-1987, I was an assistant professor at the Duke University Medical Center in the Departments of Radiology and Pharmacology.

5. From 1988-1993, I was an assistant professor and an associate professor at the Medical College of Virginia for the Virginia Commonwealth University in the Department of Pathology.

6. In 1993 I joined the faculty at University of Pittsburgh in the Department of Pathology as an associate professor and I am now a full professor.

7. During my 35+ years of experience associated with the research and study of hepatocytes, I have authored over 160 publications, edited/authored 18 chapters of reference books, and lectured numerous times at seminars and industry meetings throughout the world.

8. During my tenure as a professor, I have also consulted several companies including Becton Dickinson and Company, Rohm & Haas, Pharmacia Upjohn, Stemnion, and Cambrex-Lonza.

9. I have taught courses to graduate students in areas involving: stem cells; molecular mechanisms of tissue growth and differentiation; cell structure, metabolism, and nutrition; cancer biology and therapeutics; and pathobiology.

10. I have received the National Cancer Institute Fellowship from the National Heath Institutes of Health ("NIH"). Since 2000, I have served as the Editor for *Hepatocytes* for the Journal of Cell Transplantation, which is one of the two most authoritative transplant references in the field.

11. My research interests include hepatocyte transplantation as a clinical treatment of liver disease, expression and regulation of drug-metabolizing enzymes and transporters in human liver and the production of stem or progenitor cells, particularly from amnion.

12. My laboratory focuses on isolation culture and transplantation of human hepatocytes and their use in basic an clinical research. My laboratory was the first to isolate and transplant normal human hepatocytes from cadaver donors into patients to treat liver disease. My laboratory was also the first lab approved by the FDA for the isolation of human hepatocytes for clinical transplant and I have an active IND for these clinical hepatocyte transplants.

13. My laboratory is presently funded by NIH as a national resource to provide isolated human hepatocytes to NIH-funded investigators. In that capacity my laboratory has provided over 20,000 plates or flasks of human hepatocytes to investigators. I have published extensively on the use of human hepatocytes for studying CYP450 gene expression and drug metabolism. I have been investigating alternative stem cell sources that might provide cells for clinical hepatocyte transplants, including those from human amnion (membrane which builds the amniotic sac that protects an embryo). My current work focuses on making iPS cells from

patients with metabolic disease, and the possibility that the genetically-corrected cells could be differentiated back to hepatocytes for clinical transplant.

14. I have also won a number of awards associated with my teaching and research, including being awarded numerous financial research grants from the National Heath Institutes of Health which add into the millions.

B. Compensation

15. I am being compensated at an hourly rate of $200 per hour, plus reasonable expenses. My compensation does not depend in any way on the outcome of this case.

C. Prior Testimony

16. I have not provided any expert witness testimony within the past four years.

17. Attached as Exhibit A is a true and accurate copy of my *curriculum vitae* as of the date of this declaration.

II. ASSIGNMENT AND DOCUMENTS REVIEWED

18. Celsis IVT's counsel asked me review U.S. Patent No. 7,604,929 B2 ("the '929 patent")[1]. I specifically reviewed the claims, specification, and prosecution history of the '929 patent and the references cited on the face of the '929 patent.

19. Celsis IVT's counsel also asked me to interpret certain terms appearing in Claims 1, 3-5, 7, and 10 of the '929 patent, as they would have been understood by a person of ordinary skill in the art.

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[1] Exhibit C (U.S. Patent No. 7,604,929 B2).

20. Celsis IVT's counsel also asked me to provide a general overview of the basic scientific principles contemplated by the subject matter of the '929 patent.

21. Celsis IVT's counsel further asked me to review certain documents related CellzDirect's hepatocyte products.

22. Celsis IVT's counsel also asked me to determine whether the process CellzDirect uses to produce its multi-cryopreserved hepatocyte products contains each and every term in each of the Claims 1, 3-5, 7, and 10 of the '929 patent as I believe a person of ordinary skill in the art would interpret them.

23. Celsis IVT's counsel additionally asked me to review the references in the prosecution history of the '929 patent and determine whether a person of ordinary skill in the art would understand these references as collectively disclosing the processes in the '929 patent.

24. I provide as Exhibit B a list of documents that I reviewed in forming my opinions in this Declaration.

III. SCIENTIFIC PRINCIPLES REGARDING CRYOPRESERVED HEPATOCYTES

25. The liver generally contains four main types of cells: vascular endothelial cells (*i.e.*, blood vessel cells), bile duct cells, stellate cells (*i.e.*, support matrix cells), and hepatocytes. Among these cells hepatocytes are the most relevant here. Hepatocytes are cells derived from the liver and perform most of the functions associated with the liver. For example, hepatocytes metabolize and excrete drugs and toxins (*e.g.*, ammonia), produce essential proteins such as blood clotting factors and serum proteins (*e.g.*, albumin and anti-proteases), metabolize bilirubin, and perform a variety of other important functions. Hepatocytes comprise the bulk of the weight

of the liver and are important cells for researchers to study because of their functions explained above. As such, hepatocytes are important research tools for the pharmaceutical industry.

26. One problem with using hepatocytes for research is the general unavailability of human liver tissue. To deal with the unavailability of human liver tissue researchers began cryopreserving hepatocytes in the 1970's. Cryopreservation is the technique of storing cells in a frozen state. While there are many cryopreservation techniques, most methods include the use of liquid culture media, serums, cryoprotectants, and controlled freezing techniques. When the cells are needed for use, they are thawed and the cryoprotectant is diluted to prevent cell toxicity. The primary benefit of cryopreserving hepatocytes is that these cells are now routinely available to researchers or clinicians, whereas prior to cryopreservation, primary hepatocytes were only occasionally available.

27. One common problem with cryopreserving hepatocytes is the damage caused to the cells and the resulting significant decrease in cell viability. One technique for isolating viable hepatocytes from the damaged cells is the use of a density gradient. The use of a density gradient effectively separates damaged hepatocytes and other cellular debris from intact hepatocytes by their respective density. The density gradient procedure generally involves centrifuging a cell suspension through a density gradient medium (*e.g.*, Percoll® or sucrose) to form a cell pellet. The resulting cell pellet typically contains predominately viable hepatocytes, while the damaged cells, cellular debris, and non-parenchymal cells remain suspended in the density gradient medium. The hepatocytes in the cell pellet are then resuspended for use.

IV. BASES OF MY OPINIONS

A. A Person of Ordinary Skill in the Art at the Time of the Invention

28. I have reviewed and understand the '929 patent.

29. I understand from Celsis IVT's counsel that the time of the invention of the '929 patent is at least April 21, 2005, the filing date of the patent application that eventually issued as the '929 patent.

30. I have been told to consider the following factors in identifying the experience and knowledge of a person having "ordinary skill" in this art: (1) the educational level of the inventor; (2) the type of problems encountered in the art; (3) prior art solutions to those problems; (4) the rapidity with which innovations are made; (5) the sophistication of the technology; and (6) educational level of active workers in the field.

31. For the first factor, Celsis IVT's counsel provided me the Declaration of Daniel Dryden[2], which discloses that both inventors possessed a bachelors degree in chemistry and at least two years experience with cryopreserving hepatocytes. As for the other factors two through six, I am generally familiar with each of these based upon my experiences teaching, consulting, and researching in the field.

32. Based upon my understanding of the '929 patent and the prior art at the time of the invention, I believe that a person of ordinary skill in the art is a person who holds at least a bachelor of science in chemistry, biochemistry, cellular biology, or the like and has one to three years of work experience in the field with primary human hepatocytes. In addition, a person of ordinary skill in the art would consult his colleagues having expertise in the area of

[2] Declaration of Daniel Dryden dated June 21, 2010 ("Dryden Declaration").

cryopreservation of hepatocytes and/or read the published literature concerning the same, should that person find himself over his head.

B. The Parties' Processes and Products

33. I think it is helpful to discuss the parties' respective processes used to produce the relevant multi-cryopreserved hepatocyte products.

1. Manufacturing Celsis IVT's LiverPool™ Products

34. I understand from the Dryden Declaration that Celsis IVT markets and sells a product called LiverPool™ which is made according to the '929 patent.[3] I also understand that the LiverPool™ products are created from multiple donors (*i.e.*, different liver tissues) which are pooled together and then cryopreserved.

2. Manufacturing the CellzDirect Cryopreserved Hepatocyte Products

35. CellzDirect markets and sells many different types of cryopreserved hepatocyte products.[4] One of those products is a multiple donor pooled cryopreserved human hepatocyte product bearing the catalog name HMCH-M8-P10.[5] This catalog item includes at least four different lots: HuP50, HuP58, HuP59, and HuP60.[6]

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[3] Dryden Declaration at ¶ 10.

[4] Exhibit E.2.

[5] Exhibit E.1.

[6] Exhibit E.1.

36. CellzDirect has engaged Advanced Pharmaceutical Sciences, Inc. ("APS") to produce its human cryopreserved human hepatocytes.[7] APS exclusively produces single, pooled, and plateable cryopreserved hepatocytes for CellzDirect.[8]

37. In general, the process APS uses to produce the pre-pooled cryopreserved hepatocyte products for CellzDirect includes the steps of cryopreserving individual donor lots, thawing these lots, pooling them, and then re-cryopreserving them.[9] For example, HuP58, a human 10-donor pool (5 male; 5 female), is a pre-pooled twice-cryopreserved hepatocyte product sold by CellzDirect that was produced using the process performed by APS.[10] HuP58 exhibits an initial viability of 81% and a 71% viability after two hours at 37° C, as determined using a Trypan Blue exclusion method.[11] When tested for certain enzymatic activities, HuP58 exhibits a metabolic profile, including ECOD, 7-HCG/S, CYP 1A2, CYP 2C8, CYP 2C9, CYP 2C19, CYP 2D6, and CYP 3A4.[12] According to CellzDirect, HuP58 is useful in *in vitro* drug metabolism studies, such that the results of a single assay would represent the average of multiple individuals.[13]

C. The Meaning of the Claim Terms in the '929 Patent

1. The meaning of the '929 patent claim terms to a person of ordinary skill in the art

38. I discuss below the meaning of certain claim terms stated Claims 1, 3-5, 7, and 10 of the '929 patent, as would have been understood by a person of ordinary skill in the art at the time of

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[7] Exhibit E.5.

[8] Exhibits E.4 and E.5.

[9] Exhibit E.6 at p. 427-428; *see also* E.1 and E.9.

[10] Exhibit E.6 at p. 427-428; *see also* E.1 and E.9.

[11] Exhibit E.6. at p. 428 (Figure 13.1); *see also* E.1.

[12] Exhibit E.6. at p. 428 (Figure 13.1); *see also* E.1.

[13] Exhibit E.6 at p. 427; *see also* E.9.

the invention. Celsis IVT's counsel has informed me that to determine the meaning a claim term, I must first determine the plain and ordinary meaning of the claim term, examine the claim term in the context of the claim itself, consult the specification, and then consult the prosecution history for any inconsistencies with the meaning that I have determined. I have provided a detailed description of my analysis as Exhibit D.

a. The meaning of the claim terms in Claim 1 of the '929 patent

(A) "preparation of multi-cryopreserved hepatocytes"

39. Based upon my review of the claims, specification, and prosecution history of the '929 patent, a person of ordinary skill in the art would interpret the claim term "preparation of multi-cryopreserved hepatocytes" to mean a composition of hepatocytes that have been frozen and thawed at least two times according to the claimed process. I provide my analysis below.

(1) The plain and ordinary meaning of "preparation of multi-cryopreserved hepatocytes"

40. A person of ordinary skill in the art at the time of the invention would have understood the term "preparation of multi-cryopreserved hepatocytes" by its plain and ordinary meaning as a composition of hepatocytes that have been frozen at least two times.

(2) The claims

41. The claim language of the '929 patent, especially Claims 1, 3, 5, and 7 provides support for my interpretation for "preparation of multi-cryopreserved hepatocytes" and reads as follows:

> 1. A method of producing a desired **preparation of multi-cryopreserved hepatocytes**, said hepatocytes, being capable of being frozen and thawed at least two times, and in which greater than 70% of the hepatocytes of said preparation are viable after the final thaw, said method comprising:

> (A) subjecting hepatocytes that have been frozen and thawed to density gradient fractionation to separate viable hepatocytes from non-viable hepatocytes,
>
> (B) recovering the separated viable hepatocytes, and
>
> (C) cryopreserving the recovered viable hepatocytes to thereby form said desired preparation of hepatocytes without requiring a density gradient step after thawing the hepatocytes for the second time, wherein the hepatocytes are not plated between the first and second cryopreservations, and wherein greater than 70% of the hepatocytes of said preparation are viable after the final thaw.
>
> 3. The method of claim 1, wherein said **hepatocytes** are selected from the group consisting of human hepatocytes, porcine hepatocytes, simian hepatocytes, canine hepatocytes, feline hepatocytes, bovine hepatocytes, equine hepatocytes, ovine hepatocytes and rodent hepatocytes.
>
> 5. The method of claim 1, wherein said **preparation** comprises a pooled preparation of hepatocytes of multiple sources.
>
> 7. The method of claim 5, wherein the **hepatocytes of said pooled preparation** of hepatocytes provide said pooled preparation with a desired level of a metabolic activity. (emphasis mine)[14]

42. The language of Claim 1 provides that the "preparation of multi-cryopreserved hepatocytes" is "capable of being frozen and thawed at least two times." Incidentally, I note that the claim language apparently uses the term "cryopreserved" interchangeably with "frozen", which was common in the art at the time of the invention.

43. The language of Claim 1 also provides a process by which one would create the preparation of multi-cryopreserved hepatocytes by: "(A) subjecting hepatocytes that have been frozen and thawed to density gradient fractionation to separate viable hepatocytes from non-

[14] Exhibit C at col. 19:56 - col. 20:38.

viable hepatocytes, (B) recovering the separated viable hepatocytes, and (C) cryopreserving the recovered viable hepatocytes to thereby form said desired preparation of hepatocytes without requiring a density gradient step after thawing the hepatocytes for the second time."

44. In addition, several other claims use the term "preparation of multi-cryopreserved hepatocytes" consistent with its plain and ordinary meaning. Claim 3 provides that the preparation may be human, porcine, simian, canine, feline, bovine, equine, ovine, and/or rodent hepatocytes. Claim 5 provides that the preparation may be a pool of hepatocytes of multiple sources. Claim 7 provides that the pooled preparation has a desired level of metabolic activity.

(3) The specification

45. A person of ordinary skill in the art would have read the patent specification of the '929 patent and understood that it discloses the "preparation of multi-cryopreserved hepatocytes" consistent with its plain and ordinary meaning. For example, the specification discloses that "'**multi-cryopreserved hepatocyte preparation**' denotes a hepatocyte preparation that has been frozen and thawed at least two times . . . [s]uch preparations may have been frozen and thawed three, four, five, or more times." (emphasis mine)[15]. Another example from the specification discloses that the preparations either permit for or have been subjected to repeated cryopreservation and thawing while retaining substantial viability:

> In particular, the invention concerns methods of processing **preparations** of cells, especially hepatocytes, so as to permit their repeated cryopreservation and thawing while retaining substantial viability. The invention also concerns **preparations** of cells (e.g., hepatocytes) that have been repeatedly cryopreserved and thawed. (emphasis mine)[16]

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[15] Exhibit C at col. 5:47-51; *see also* Exhibit C at col. 5:32-33 and col. 5:52-54.

[16] Exhibit C at col. 4:12-17.

46. The specification also discloses that the preparation is a composition by stating that "'hepatocyte preparation' denotes a composition of hepatocytes from one or more sources."[17]

(4) The prosecution history

47. A person of ordinary skill in the art would have read the prosecution history and understood that it does not disclose any use of the term "preparation of multi-cryopreserved hepatocytes" inconsistent with those disclosures discussed above in the claims and specification.

(B) "density gradient fractionation"

48. Based upon my review of the claims, specification, and prosecution history of the '929 patent, a "person of ordinary skill in the art" would interpret the claim term "density gradient fractionation" to mean a process for separating viable hepatocytes from non-viable hepatocytes based on their density. I provide my analysis below.

(1) The plain and ordinary meaning of "density gradient fractionation"

49. A person of ordinary skill in the art at the time of the invention would have understood the term "density gradient fractionation" by its plain and ordinary meaning as a process for separating cells, subcellular fractions, or any two components within a solution based on their density. Density is the ratio of weight/volume (w/v).

(2) The claims

50. The claim language of the '929 patent, especially Claims 1 and 2, provide support to my interpretation and read as follows:

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[17] Exhibit C at col. 5:34-35.

> 1. A method of producing a desired preparation of multi-cryopreserved hepatocytes, said hepatocytes, being capable of being frozen and thawed at least two times, and in which greater than 70% of the hepatocytes of said preparation are viable after the final thaw, said method comprising:
>
> (A) subjecting hepatocytes that have been frozen and thawed to **density gradient fractionation** to separate viable hepatocytes from non-viable hepatocytes,
>
> (B) recovering the separated viable hepatocytes, and
>
> (C) cryopreserving the recovered viable hepatocytes to thereby form said desired preparation of hepatocytes without requiring a **density gradient** step after thawing the hepatocytes for the second time, wherein the hepatocytes are not plated between the first and second cryopreservations, and wherein greater than 70% of the hepatocytes of said preparation are viable after the final thaw.
>
> 2. The method of claim 1, wherein said **density gradient fractionation** comprises density centrifugation through polyvinylpyrrolidone-coated colloidal silica particles. (emphasis mine)[18]

51. A person of ordinary skill in the art would have understood Claim 1 as requiring a density gradient fractionation step to separate viable hepatocytes from non-viable hepatocytes between the first and second cryopreservation, but would not have read the language to require any specific method for performing the density gradient fractionation. Claim 1 does not require a particular type density gradient fractionation. Claim 2 only requires that the density gradient fractionation step be performed using polyvinylpyrrolidone-coated colloidal silica particles, though there are other known alternatives. Claim 2 would tell to a person of ordinary skill in the art that a "density gradient fractionation" step would not necessarily include a wash step to remove, for example, the cryoprotectant after thawing. Accordingly, a person of ordinary skill in the art would understand that the claims disclose the term "density gradient fractionation" to

[18] Exhibit C at col. 19:56 – col. 20:23.

mean a process for separating cells, subcellular fractions, or any two components within a solution based on their density, which is consistent with its plain and ordinary meaning.

(3) The specification

52. A person of ordinary skill in the art would have read the patent specification of the '929 patent and have understood that it discloses the term "density gradient fractionation" consistent with that its plain and ordinary meaning. For example, the specification discloses density gradient fractionation as a favorable method for separating viable and non-viable cells.[19] The specification also discloses several different embodiments for performing this separation step including the use of Percoll® or Percoll/Redigrad®.[20]

(4) The prosecution history

53. A person of ordinary skill in the art would have read the prosecution history and have understood that it does not disclose any use of the term "density gradient fractionation" inconsistent with those disclosures discussed above in the claims and specification.

(C) "without requiring a density gradient fractionation step after thawing the hepatocytes for a second time"

54. Based upon my review of the claims, specification, and prosecution history of the '929 patent, a person of ordinary skill in the art would interpret the claim term "without requiring a density gradient fractionation step after thawing the hepatocytes for a second time" to mean that the claimed process does not require a density gradient fractionation step after thawing the hepatocytes a second time. I provide my analysis below.

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[19] Exhibit C at col. 10:30-33.

[20] Exhibit C at col. 10:33-35, col. 14:22-54, and col. 17:60-67.

(1) The plain and ordinary meaning

55. A person of ordinary skill in the art at the time of the invention would have understood the term "without requiring a density gradient fractionation step after thawing the hepatocytes for a second time" by its plain and ordinary meaning that the claimed process does not require a density gradient fractionation step after thawing the hepatocytes a second time.

(2) The claims

56. The language of Claim 1 of the '929 patent provides support to my interpretation and reads as follows:

> 1. A method of producing a desired preparation of multi-cryopreserved hepatocytes, said hepatocytes, being capable of being frozen and thawed at least two times, and in which greater than 70% of the hepatocytes of said preparation are viable after the final thaw, said method comprising:
>
> (A) subjecting hepatocytes that have been frozen and thawed to density gradient fractionation to separate viable hepatocytes from non-viable hepatocytes,
>
> (B) recovering the separated viable hepatocytes, and
>
> (C) cryopreserving the recovered viable hepatocytes to thereby form said desired preparation of hepatocytes **without requiring a density gradient step after thawing the hepatocytes for the second time**, wherein the hepatocytes are not plated between the first and second cryopreservations, and wherein greater than 70% of the hepatocytes of said preparation are viable after the final thaw. (emphasis mine)[21]

57. A person of ordinary skill in the art would have read the language of Claim 1 and have understood that "without requiring" means that to practice the claimed process one does not need to perform the step of subjecting the preparation to "a density gradient fractionation" after

[21] Exhibit C at col. 19:56 – col. 20:20.

thawing the hepatocytes a second time in order to achieve the "desired preparation of hepatocytes."

(3) The specification

58. A person of ordinary skill in the art would have read the specification of the '929 patent and have understood that it discloses "without requiring ...." consistent with that its plain and ordinary meaning. I note that the specification does not disclose the presence of this step as necessary in order to achieve the desired hepatocyte preparation as required by the claims (*e.g.*, "*by requiring* a density gradient fractionation step after thawing the hepatocytes for a second time.")

(4) The prosecution history

59. A person of ordinary skill in the art would have read the prosecution history of the '929 patent and have understood that it discloses the term "without requiring . . ." consistent with its plain and ordinary meaning.

(a) The claims are not "obvious" over prior art

60. The Applicants submitted a Reply responsive to the Examiner's Office Action dated March 18, 2008, and the personal interview dated August 6, 2008.[22] To overcome the "obviousness" rejection over Shibata and Ostrowska, the Applicants amended then-pending Claim 10 (which issued as Claim 1) by adding the language "without requiring a density gradient step after thawing the hepatocytes for the second time, wherein the hepatocytes are not plated

[22] Exhibit F at Tab 22 at p. 6 (Reply to Office Action dated 8-15-08).

between the first and second cryopreservations."[23] The Applicants explained that "the present invention does not require a Percoll density gradient to remove non-viable cells, while retaining metabolic activity and viability over 70%, which is required by the claims."[24] The Applicants further explained that "a person of ordinary skill in the art would have not had any expectation of success based on the disclosures in the cited references, much less a reasonable one, that hepatocytes could be multiply-cryopreserved, so as to maintain high viability of over 70%, without requiring a second Percoll density gradient step or plating the hepatocytes between cryopreservation steps."[25] I agree with the Applicant's explanation of the understanding of a person of ordinary skill in the art that the invention is not "obvious." I understand that an invention is "obvious" when a person of ordinary skill in the art would want to piece together prior art references in order to create the invention.

61. In response to the Applicants' Reply dated August 15, 2008, the Examiner withdrew her prior "obviousness" rejection of then-pending Claim 10 over Shibata in combination with Ostrowska.[26]

(b) The claim language is not "new matter"

62. The Examiner then issued a new rejection of then-pending Claim 10, because she considered the language "without requiring a density gradient step after thawing the hepatocyte for the second time" to be "new matter."[27] The Examiner explained that "[t]he instant specification and the original claims only provide support for a method of preparing multi-

[23] Exhibit F at Tab 22 at p. 3 (Reply to Office Action dated 8-15-08).

[24] Exhibit F at Tab 22 at p. 10 (Reply to Office Action dated 8-15-08).

[25] Exhibit F at Tab 22 at p. 10 (Reply to Office Action dated 8-15-08).

[26] Exhibit F at Tab 21 at p. 2 (Office Action dated 11-13-08).

[27] Exhibit F at Tab 21 at p. 16 (Office Action dated 11-13-08).

cryopreserved hepatocytes by using Percoll density gradient centrifugation after each thawing, wherein such density gradient centrifugation after each thawing removes the non-viable cells."[28] Based upon my understanding of "new matter", which is when subject matter appearing in the claim language does not appear in the specification, I disagree with the Examiner. The examples in the specification do not include the step of using a density gradient step after thawing the hepatocyte for the second time.

63. The Examiner also issued a new rejection of then-pending Claim 10, because of a "lack of enablement."[29] The Examiner further explained that "the instant specification only teaches successfully obtaining multi-cryopreserved hepatocytes by using Percoll centrifugation after each cryopreservation/thawing cycle. There is no teaching in the specification regarding obtaining such preparation without a Percoll centrifugation step after the second thawing."[30] Based upon my understanding of "lack of enablement," which is when a person of ordinary skill in the art would not be able to practice the invention without using excessive efforts, I disagree with the Examiner. The examples in the specification do not require using Percoll centrifugation after *each* cryopreservation/thawing cycle to successfully obtain multi-cryopreserved hepatocytes. In absence of a requirement to the contrary, a person of ordinary skill in the art would obviously know how to refrain from performing this step.

64. The Applicants responded to the Examiner's "new matter" rejection by explaining that the specification contained sufficient written support for the claim amendment:

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[28] Exhibit F at Tab 21 at p. 17 (Office Action dated 11-13-08).

[29] Exhibit F at Tab 21 at p. 17-20 (Office Action dated 11-13-08).

[30] Exhibit F at Tab 21 at p. 19 (Office Action dated 11-13-08).

> While Applicants understand the premise that Percoll density gradient centrifugation will remove non-viable cells, the claims require only that Percoll density gradient centrifugation is performed *prior* to re-freezing. For example, claim 1 recites "[a] multi-cryopreserved hepatocyte preparation comprising hepatocytes that have been frozen and thawed at least two times, wherein greater than 70% of the hepatocytes of said preparation are viable without requiring a density gradient step after thawing the hepatocytes for the second time..." In addition, the specification does not disclose that a Percoll™ density gradient centrifugation step is required *after* the final thawing. For example, paragraphs [0015] – [0017] illustrate a three step protocol, in which a Percoll™ density gradient centrifugation step is found in step (A), which is *prior* to a cryopreservation step. The specification does not disclose a Percoll™ density gradient centrifugation step *after* thawing the cells for the *second, or final* time. Also, originally filed claim 10 also recites the same steps, *without* requiring a Percoll™ density gradient centrifugation step after the *final* thawing.
>
> Applicants amended the claims to recite that a Percoll™ density gradient centrifugation step after a final thawing is not required. Negative provisos may carve out a specific prior art disclosure in order to "merely excis[e] the invention of another." *In re Johnson*, 558 F.2d 1008, 1019 (C.C.P.A. 1977).
>
> In view of these factors, the claimed invention has written support in the specification, and Applicants believe that they have overcome the Examiner's rejection. (emphasis in original)[31]

65. Regarding the "lack of enablement" rejection, the Applicants explained that in the face of the unpredictable hepatocyte art, "there is no teaching in the specification or a recited limitation in the claims that a Percoll™ density gradient centrifugation step after thawing a multi-

[31] Exhibit F at Tab 20 at p. 17 (Reply Office Action dated 2-12-09).

cryopreserved hepatocyte product is required. Rather, this step *is only required before* a hepatocyte culture undergoes cryopreservation." (emphasis in original)[32]

66. In response to the Applicants' Reply dated February 12, 2009, the Examiner maintained her "new matter" and "lack of enablement" rejections for essentially the same reasons she stated in her Office Action dated November 13, 2008.[33]

67. In response to the Examiner's Final Office Action dated May 12, 2009, and the personal interview on June 2, 2009, the Applicants amended then-pending Claim 10 to include the language "after the final thaw" and "wherein greater than 70% of the hepatocytes of said preparation are viable after the final thaw."[34] Regarding both the "new matter" and "lack of enablement" rejections, the Applicants understood that the Examiner had withdrawn them during the personal interview.[35]

68. Finally, the Examiner withdrew her prior "new matter" and "lack of enablement" rejections for then-pending Claims 10-17, 19, 20, and 22,[36] and allowed those claims to issue as Claims 1-11 of the '929 patent on October 20, 2009.[37]

(D) "plated"

69. Based upon my review of the claims, specification, and prosecution history of the '929 patent, a person of ordinary skill in the art would interpret the claim term "plated" to mean to

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[32] Exhibit F at Tab 20 at p. 18 (Reply Office Action dated 2-12-09).

[33] Exhibit F at Tab 19 at p. 1-19 (Final Office Action dated 5-12-09).

[34] Exhibit F at Tab 17 at p. 9, 12 (Reply to Office Action dated 5-22-09).

[35] Exhibit F at Tab 17 at p. 17, 18 (Reply to Office Action dated 5-22-09).

[36] Exhibit F at Tab 16 at p. 2 (Advisory Action dated 7-16-09).

[37] Exhibit F at Tab 13 at p. 1-4 (Notice of Allowability dated 8-20-09).

have placed hepatocytes on a laboratory plate containing attachment substrates (*e.g.*, collagen or extra-cellular matrix proteins). I provide my analysis below.

(1) The plain and ordinary meaning of "plated"

70. A person of ordinary skill in the art at the time of the invention would have understood the term "plated" by its plain and ordinary meaning to mean to have placed cells on a laboratory plate containing attachment substrates. The plain and ordinary meaning of "plated" would be applicable to any type of cell that is susceptible to being plated.

(2) The claims

71. The language of Claim 1 of the '929 patent provides support to my interpretation and reads as follows:

> 1. A method of producing a desired preparation of multi-cryopreserved hepatocytes, said hepatocytes, being capable of being frozen and thawed at least two times, and in which greater than 70% of the hepatocytes of said preparation are viable after the final thaw, said method comprising:
>
> (A) subjecting hepatocytes that have been frozen and thawed to density gradient fractionation to separate viable hepatocytes from non-viable hepatocytes,
>
> (B) recovering the separated viable hepatocytes, and
>
> (C) cryopreserving the recovered viable hepatocytes to thereby form said desired preparation of hepatocytes without requiring a density gradient step after thawing the hepatocytes for the second time, **wherein the hepatocytes are not plated between the first and second cryopreservations**, and wherein greater than 70% of the hepatocytes of said preparation are viable after the final thaw. (emphasis mine)[38]

[38] Exhibit C at col. 19:56 – col. 20:20.

72. A person of ordinary skill in the art would have read Claim 1 and have understood that the "plated" step does not occur between the first and second cryopreservations. The "plated" step could occur after at least the second cryopreservation. Further, Claim 1 requires that the "plated" cells be hepatocytes, as opposed to some other type of cell.

(3) The specification

73. A person of ordinary skill in the art would have read the '929 patent specification and understood that it discloses "plated" hepatocytes and the plating step consistent with that its plain and ordinary meaning. For example, the specification discloses plating the hepatocytes after a second cryopreservation using collagen-coated tissue culture plates or tissue culture plates coated with other extra-cellular matrix proteins, including laminin, fibronectin, entactin, poly-L-lysine, gelatin, or any combination of the same.[39]

(4) The prosecution history

74. A person of ordinary skill in the art would have read the '929 patent prosecution history and have understood that it does not disclose any use of the term "plated" inconsistent with those disclosures discussed above in the claims and specification.

(E) "the hepatocytes of said preparation are viable after the final thaw"

75. Based upon my review of the claims, specification, and prosecution history of the '929 patent, a person of ordinary skill in the art would interpret the claim term "the hepatocytes of said preparation are viable after the final thaw" to mean the percentage of living hepatocytes relative to the total cell population in the preparation, when determined after the final thaw. I provide my analysis below.

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[39] Exhibit C at col. 13:25-43; *see also* col. 19:17-24.

(1) The plain and ordinary meaning of "the hepatocytes of said preparation are viable after the final thaw"

76. A person of ordinary skill in the art at the time of the invention would have understood the term "the hepatocytes of said preparation are viable after the final thaw" to mean the percentage of living hepatocytes relative to the total cell population in the preparation, when determined after the final thaw.

(2) The claim

77. The language of Claim 1 provides support to my interpretation and reads as follows:

> 1. A method of producing a desired preparation of multi-cryopreserved hepatocytes, said hepatocytes, being capable of being frozen and thawed at least two times, and in which greater than 70% of **the hepatocytes of said preparation are viable after the final thaw**, said method comprising:
>
> (A) subjecting hepatocytes that have been frozen and thawed to density gradient fractionation to separate **viable hepatocytes** from non-viable hepatocytes,
>
> (B) recovering the separated viable hepatocytes, and
>
> (C) cryopreserving the recovered viable hepatocytes to thereby form said desired preparation of hepatocytes without requiring a density gradient step after thawing the hepatocytes for the second time, wherein the hepatocytes are not plated between the first and second cryopreservations, and **wherein greater than 70% of the hepatocytes of said preparation are viable after the final thaw**. (emphasis mine)[40]

78. A person of ordinary skill in the art would have understood from the claim language itself that 70% of the hepatocytes must be viable when determined after the final thaw.

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[40] Exhibit C at col. 19:56 – col. 20:20.

(3) The specification

79. A person of ordinary skill in the art at the time of the invention would have read the '929 patent specification and understood that it discloses the term "the hepatocytes of said preparation are viable after the final thaw" consistent with its plain and ordinary meaning. The '929 patent specification discloses that "[i]n particular, the invention concerns methods of processing preparations of cells, especially hepatocytes, so as to permit their repeated cryopreservation and thawing while retaining **substantial viability**."[41] (emphasis mine).

80. The specification also discloses various methods for determining cell viability: "The viability of the isolated hepatocytes may be determined using any of a variety or methods. Preferably, such viability will be determined using the Trypan blue exclusion method ...Thus the phrases 'viable hepatocytes' or 'percent viability,' as used herein, refers to hepatocyte viability as assessed using the method of Trypan Blue exclusion."[42] A person of ordinary skill in the art would have read this disclosure and have understood it to mean that hepatocyte viability may be determined using a variety of methods, but that the preferred method is using the Trypan Blue exclusion method.

81. The specification further discloses the percentage of cell viability: "Cryopreserved preparations that result from the freezing of a previously frozen-thawed preparation will preferably have a post-thaw cell viability of greater than 50% and more preferably 70% or more.[43]

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[41] Exhibit C at col. 4:12-15; *see also id.* at col. 5:11-15.

[42] Exhibit C at col. 7:22-30; *see also id.* at col. 9:54-55.

[43] Exhibit C at col. 10:61-64.

(4) The prosecution history

82. A person of ordinary skill in the art would have read the '929 patent prosecution history and understood that it does not disclose any use of the term "the hepatocytes of said preparation are viable after the final thaw" inconsistent with those disclosures discussed above in the claims and specification.

b. The meaning of the claim terms in Claim 10 of the '929 patent.

83. I incorporate my opinions and analysis for those terms appearing in Claim 1, as explained above, into my analysis here regarding Claim 10.

(A) "incubating"

84. Based upon my review of the claims, specification, and prosecution history of the '929 patent, a person of ordinary skill in the art would interpret the claim term "incubating" to mean simulating certain biological conditions. I provide my analysis below.

(1) The plain and ordinary meaning of "incubating"

85. A person of ordinary skill in the art at the time of the invention would have understood the term "incubating" to mean simulating certain biological conditions.

(2) The claims

86. The language of Claim 10 provides support to my interpretation and reads as follows:

> 10. A method of investigating in vitro drug metabolism comprising **incubating hepatocytes of a multi-cryopreserved hepatocyte preparation in the presence of a xenobiotic**, and determining the metabolic fate of the xenobiotic, or the affect of the xenobiotic on the hepatocytes or on an enzyme or metabolic activity thereof, wherein the hepatocytes have been frozen and thawed at least two times, and wherein greater than 70% of the hepatocytes of said preparation are viable without requiring

> a density gradient step after thawing the hepatocytes for the second time, wherein the hepatocytes are not plated between the first and second cryopreservations. (emphasis mine)[44]

87. A person of ordinary skill in the art would have understood that "incubating" of the multi-cryopreserved hepatocyte preparation must be in the presence of a xenobiotic.

(3) The specification

88. A person of ordinary skill in the art at the time of the invention would have read the '929 patent specification and have understood that it discloses the term "incubating" consistent with its plain and ordinary meaning. For example, the '929 patent specification discloses "incubating" at certain conditions:

> A common use of for cryopreserved hepatocytes is to thaw the hepatocytes and then incubate them with various xenobiotics. For this purpose, it is preferred that the hepatocytes maintain their viability for at least several hours. To examine the post-thaw viability over time for one lot of pooled cryopreserved hepatocytes, the cells were thawed, aliquoted into the wells of a 12-well plate, and **incubated** at 37° C. with 5% CO_2. (emphasis mine)[45]

89. A person of ordinary skill in the art would have also understood a variety of different conditions upon which "incubating" could occur.

(4) The prosecution history

90. A person of ordinary skill in the art would have read the '929 patent prosecution history and have understood that it does not disclose any use of the term "incubating" inconsistent with those disclosures discussed above in the claims and specification.

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[44] Exhibit C at col. 20:50-60.

[45] Exhibit C at col. 19:17-24; *see also id.* at col. 4:66-67.

(B) "xenobiotic"

91. Based upon my review of the claims, specification, and prosecution history of the '929 patent, a person of ordinary skill in the art would interpret the claim term "xenobiotic" to mean a substance foreign to the body. I provide my analysis below.

(1) The plain and ordinary meaning of "xenobiotic"

92. A person of ordinary skill in the art at the time of the invention would have understood the term to mean a substance foreign to the body.

(2) The claims

93. The language of Claim 10 provides support to my interpretation and reads as follows:

> 10. A method of investigating in vitro drug metabolism comprising incubating hepatocytes of a multi-cryopreserved hepatocyte preparation **in the presence of a xenobiotic**, and determining **the metabolic fate of the xenobiotic**, or the **affect of the xenobiotic on the hepatocytes** or on an enzyme or metabolic activity thereof, wherein the hepatocytes have been frozen and thawed at least two times, and wherein greater than 70% of the hepatocytes of said preparation are viable without requiring a density gradient step after thawing the hepatocytes for the second time, wherein the hepatocytes are not plated between the first and second cryopreservations. (emphasis mine)[46]

94. A person of ordinary skill in the art would have understood that the invention permits one to determine the metabolic fate of the "xenobiotic," the effect of the "xenobiotic" on the hepatocytes, the effect of the "xenobiotic" on an enzyme, or the effect of the "xenobiotic" on the metabolic activity of the enzyme. Each use of the term "xenobiotic" in Claim 10 is consistent with its plain and ordinary meaning.

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[46] Exhibit C at col. 20:50-60.

(3) The specification

95. A person of ordinary skill in the art at the time of the invention would have read the '929 patent specification and understood that it discloses the term "xenobiotic" consistent with its plain and ordinary meaning. For example, the '929 patent specification discloses the invention "comprising incubating hepatocytes of a multi-cryopreserved hepatocyte preparation in the presence of a xenobiotic."[47]

96. The specification of the '929 patent also discloses incubating the hepatocytes with various xenobiotics:

> A common use of for cryopreserved hepatocytes is to thaw the hepatocytes and then incubate them with various **xenobiotics**. For this purpose, it is preferred that the hepatocytes maintain their viability for at least several hours. To examine the post-thaw viability over time for one lot of pooled cryopreserved hepatocytes, the cells were thawed, aliquoted into the wells of a 12-well plate, and incubated at 37° C. with 5% CO_2. The viability of the hepatocytes is then measured at time-points for up to six hours. Table VI shows the results of this analysis, wherein, at six hours, 39% of the hepatocytes remained viable. (emphasis mine).[48]

97. A person of ordinary skill in the art would have identified a typographical error in the disclosure of "39% of the hepatocytes remained viable," because Table VI discloses the actual viability as "69%." Thus, "39%" should have been "69%."

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[47] Exhibit C at col. 4:66 – col. 5:6.

[48] Exhibit C at col. 19:17-26.

(4) The prosecution history

98. A person of ordinary skill in the art would have read the '929 patent prosecution history and have understood that it does not disclose any use of the term "xenobiotic" inconsistent with those disclosures discussed above in the claims and specification.

(C) "metabolic fate"

99. Based upon my review of the claims, specification, and prosecution history of the '929 patent, a person of ordinary skill in the art would interpret the claim term "metabolic fate" to mean the modification of the chemical structure or the localization of the xenobiotic by the hepatocytes.

(1) The plain and ordinary meaning of "metabolic fate"

100. A person of ordinary skill in the art at the time of the invention would have understood the term "metabolic fate" to mean the modification of the chemical structure or the localization of the xenobiotic by the hepatocytes.

(2) The claims

101. The language of Claim 10 provides support to my interpretation and reads as follows:

> 10. A method of investigating in vitro drug metabolism comprising incubating hepatocytes of a multi-cryopreserved hepatocyte preparation in the presence of a xenobiotic, and determining **the metabolic fate of the xenobiotic**, or the affect of the xenobiotic on the hepatocytes or on an enzyme or metabolic activity thereof, wherein the hepatocytes have been frozen and thawed at least two times, and wherein greater than 70% of the hepatocytes of said preparation are viable without requiring a density gradient step after thawing the hepatocytes for the second time, wherein the hepatocytes are not plated

> between the first and second cryopreservations. (emphasis mine)[49]

102. A person of ordinary skill in the art would have understood that the invention in Claim 10 permits the determination of the "metabolic fate" of the xenobiotic (*i.e.*, determining the modification of the chemical structure or the localization of the xenobiotic by the hepatocytes).

(3) The specification

103. A person of ordinary skill in the art at the time of the invention would have read the '929 patent specification and understood that it discloses the term "metabolic fate" consistent with its plain and ordinary meaning. For example, the '929 patent specification discloses studies designed to assess the metabolic fate of the xenobiotic:

> In particular, multi-cryopreserved hepatocyte preparations may be used in in vitro drug metabolism studies (for example, in identifying hepatocytes with unique characteristics (e.g.,. metabolic polymorphisms, genetic polymorphisms, etc.) in studies on the **metabolic fate** of the xenobiotic and studies on the affect of the xenobiotic in altering the drug-metabolizing enzyme profile of the hepatocytes, in inhibition studies to determine the IC_{50} of xenobiotics on liver enzymes and functions (e.g. cholesterol metabolism), in gene induction studies with xenobiotics, in protein induction studies with xenobiotics, in toxicity assessment of xenobiotics on hepatocytes, transport studies with xenobiotics (e.g. studies on P-glycoprotein transport systems, organic ion transporters, organic cation transporters, etc.), in metabolic clearance studies with xenobiotics, and in efficacy assays (e.g. lipoprotein processing, gluconeogenesis, protein secretion etc.). (emphasis mine)[50]

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[49] Exhibit C at col. 20:50-60.

[50] Exhibit C at col. 9:67 - col. 10:16; *see also id.* at col. 18:22-65.

(4) The prosecution history

104. A person of ordinary skill in the art would have read the '929 patent prosecution history and have understood that it does not disclose any use of the term "metabolic fate" inconsistent with those disclosures discussed above in the claims and specification.

D. Comparison of the CellzDirect Process to the Claims of the '929 Patent.

105. Celsis IVT's counsel asked me to compare the CellzDirect process used to make its multiple donor pooled cryopreserved hepatocyte products, as exemplified by HuP58, to Claims 1, 3-5, 7, and 10 of the '929 patent. After completing this task, I have concluded that the process CellzDirect uses to produce HuP58 satisfies each claim term appearing in Claims 1, 3-5, 7, and 10 of the '929 patent. My analysis is attached Exhibit E. If the process CellzDirect uses to produce HuP58 is the same as or substantially similar to the process used to produce CellzDirect's other multiple donor pooled cryopreserved hepatocyte products (*e.g.*, Hu0539-3, HuP2001-03, HuP53, HuP88, HuP89, HuP94; HMCS3L, and CyP10), then my opinions and analysis apply to those similarly-produced products as well, and those processes would also satisfy each claim term appearing in Claims 1, 3-5, 7, and 10 of the '929 patent.

E. The References in the Prosecution History of the '929 Patent

106. Celsis IVT's counsel asked me to review the references in the prosecution history of the '929 patent and determine whether a person of ordinary skill in the art would understand these references as collectively disclosing the processes in the '929 patent.

107. Based upon my review of these references, including Ostrowska[51] and Shibata[52], in my opinion a person of ordinary skill in the art would understand that none of these references individually disclose the processes in the '929 patent. In addition, a person of ordinary skill in the art would not have been prompted to piece together these references to collectively create the processes in the '929 patent. For example, Ostrowska discloses that after only one cryopreservation-thaw cycle, the hepatocyte preparation will experience an average drop in viability of approximately 17%. This drop would suggest that multiple cryopreservation-thaw cycles of these fragile hepatocytes would not have resulted in hepatocytes with an average viability of greater than 70%. With Ostrowska in mind, a person of ordinary skill in the art would have not wanted to piece together steps from the other references to collectively create the process in the '929 patent. In other words, a person of ordinary skill in the art would know that these references do not collectively disclose the processes in the '929 patent.

108. If the Court adopts a modified or different version of my interpretations, then I reserve the right to comment upon those interpretations, and provide additional analysis and opinions relating to whether the process CellzDirect uses to produce its multi-cryopreserved hepatocyte products contains each of the claim terms stated in Claims 1, 3-5, 7, and 10 of the '929 patent.

Dated: June 25, 2010 By: Stephen Strom

Stephen C. Strom, Ph.D.

6/25/10

[51] Exhibit E.12 (Ostrowska, A., *et al.*, *Investigation of Functional and Morphological Integrity of Freshly Isolated and Cryopreserved Human Hepatocytes*, Cell and Tissue Banking 1 p. 55-68 (2000).

[52] Exhibit E.14 (Shibata, A., *et al.*, *Prediction of Hepatic Clearance and Availability by Cryopreserved Human Hepatocytes: An Application of Serum Incubation Method*, Drug Metabolism and Disposition vol. 30:8, p. 892-896 (2002).